REVIEW

# Infections with the tick-borne bacterium *Candidatus* Neoehrlichia mikurensis

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## Abstract

*Candidatus* Neoehrlichia mikurensis, which has rodents as its natural hosts, is an emerging tick-borne pathogen in Europe and Asia. This intracellular bacterium causes the infectious disease neoehrlichiosis. Immunocompromised patients may contract a severe form of neoehrlichiosis with high fever and vascular/thromboembolic events. As it is not detected with routine culture-based methods, neoehrlichiosis is underdiagnosed.



**Keywords:** *Candidatus* Neoehrlichia mikurensis, immunocompromised, neoehrlichiosis, rodents, tick-borne infection
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## Introduction

The aim of this review is to summarize the current knowledge on *Candidatus* Neoehrlichia mikurensis, a tick-borne bacterium that has recently been shown to be pathogenic for humans. The bacterium and its vectors, reservoirs, and modes of transmission, and the infectious disease neoehrlichiosis and its clinical picture, choice of diagnostics, and therapy, will be discussed.

## The agent

*C.* Neoehrlichia mikurensis is a new bacterial species within the novel genus *Neoehrlichia*, which belongs to the family *Anaplasmataceae*, order *Rickettsiales* [1]. There are six genera within the *Anaplasmataceae*: *Ehrlichia*, *Anaplasma*, *Wolbachia*, *Neorickettsia*, *Aegyptianella*, and *Neoehrlichia*. *C.* Neoehrlichia mikurensis was named on the basis of its resemblance to the genus *Ehrlichia* and the fact that it was isolated on the Japanese island of Mikura; the term '*Candidatus*' indicates that this bacterium is currently uncultivable [1]. Prior to the Japanese study, *C.* Neoehrlichia mikurensis was described, but under other alternative designations: *Ehrlichia*-like organism [2–4]; *Ehrlichia*-like sp. Schotti variant [3,5]; *Ehrlichia*-like sp. Rattus variant [6]; and *Candidatus* Ehrlichia walkerii [7]. The closest relative of *C.* Neoehrlichia mikurensis is the other species within the *Neoehrlichia* genus, *Candidatus* Neoehrlichia lotoris, the natural host of which is the raccoon [8].

*C.* Neoehrlichia mikurensis infects both invertebrates (ticks) and vertebrates (humans, rodents, and dogs). As *C.* Neoehrlichia mikurensis has not been cultivated to date, there is limited information regarding its morphology, cellular tropism, and life cycle. Like all other members of the *Anaplasmataceae* [1], *C.* Neoehrlichia mikurensis is assumed to be a Gram-negative obligate intracellular bacterium. Electronmicrographs of the tissues of infected rats have revealed rounded, pleomorphic structures of diameter 0.5–1.2 μm within the splenic sinusoids, which may be *C.* Neoehrlichia mikurensis [1]. However, as these structures were not labelled with DNA probes or antibodies, it was not formally proven that they were *C.* Neoehrlichia mikurensis, although they appeared to contain DNA and ribosomes [1].

The target cells of *C.* Neoehrlichia mikurensis infection in humans have not been identified with certainty, but the prime



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suspects are leukocytes and endothelium. Using transmission electron microscopy, Pekova *et al.* demonstrated coccoid structures within the circulating granulocytes of infected patients [9]. Although this could be taken to indicate that *C.* Neoehrlichia mikurensis infects granulocytes, it might simply reflect the normal phagocytic function of neutrophils. 'Morulae', which are the intracellular inclusions observed inside human leukocytes that are infected with *Anaplasma phagocytophilum* or *Ehrlichia chaffeensis*, have never been detected in patients with neoehrlichiosis. Regarding the endothelium, there are two case reports of *C.* Neoehrlichia mikurensis infection with associated arterial aneurysms, although, in both cases, it was not possible to determine whether *C.* Neoehrlichia mikurensis was the cause of the aneurysms [10,11]. To date, *C.* Neoehrlichia mikurensis has not been detected inside the walls of human blood vessels [11], and it has not been cultivated successfully in endothelial cell lines (or any other cell line for that matter).

## The epidemiology of *C.* Neoehrlichia mikurensis infections of ticks and rodents

*C.* Neoehrlichia mikurensis is widespread among ticks and rodents in Europe and Asia. It has been detected in Mongolia [12], China [6,12,13], Japan [1,14], Russia [4,5,15,16], the Czech Republic [9,17,18], Slovakia [19–21], Moldova [22], Hungary [23–25], Austria [18,26], Germany [11,27–32], Switzerland [33–37], Poland [38,39], Romania [40], France [28,41,42], Belgium [43], Italy [44–46], Spain [47], The Netherlands [3,42,43,48], and the Scandinavian countries [2,10,42,49–52]. It has not been detected in the UK [43,53,54], the USA, or Australia.

Most European studies have indicated that *C.* Neoehrlichia mikurensis is the third most common tick-borne human pathogen after *Borrelia* and *Rickettsia* species [33,44,55]. Estimates of the prevalence of *C.* Neoehrlichia mikurensis among ticks are shown in Table 1. *C.* Neoehrlichia mikurensis-infected ticks may be co-infected with other pathogenic species, such as *Borrelia*, *Babesia*, *Rickettsia*, and *Anaplasma* [14,26,28,31,44,49,56]. The rate of co-infection of ticks with *C.* Neoehrlichia mikurensis and *Borrelia* species is higher than predicted, and it may be more common for *C.* Neoehrlichia mikurensis to occur together with *Borrelia* than to appear alone in ticks [28,49,55]. This is probably a consequence of the feeding of ticks on rodents that are infected with both pathogens.

The prevalence of *C.* Neoehrlichia mikurensis seems to be increasing: a monthly surveillance study of infection rates of questing ticks conducted at 22 sites in The Netherlands between 2006 and 2010 revealed an increasing prevalence of *C.* Neoehrlichia mikurensis, in contrast to the stable prevalence rates of the other studied pathogens, including *Rickettsia helvetica*, *Borrelia* species, *Babesia* species, and *A. phagocytophilum* [55]. Although the first published report of this new species appeared in 1999 [3], these bacteria have been detected in a tick collection from 1960 [22].

The prevalence of *C.* Neoehrlichia mikurensis infection among rodents and ticks shows a seasonal pattern. In Germany, none of the tested rodents carried *C.* Neoehrlichia mikurensis in the period from March to May, 50% tested positive in June, and the prevalence rates peaked in August (76%) and thereafter declined to 36% in November [31]. In Sweden, *C.* Neoehrlichia mikurensis infection among bank voles followed the same pattern: a monthly increase in infection rate was noted from May (8.7%) and in subsequent months, reaching a maximum in September (54%) [56]. Similarly, the highest rate of *C.* Neoehrlichia mikurensis infection of ticks was seen in October in The Netherlands [55].

Studies on the genetic diversity of *C.* Neoehrlichia mikurensis have mainly been focused on the 16S rRNA and *gro*EL genes [11,12,15,35,39]. Comparative analyses of the deposited nucleotide sequences of *C.* Neoehrlichia mikurensis indicate that the genotypes circulating in Asia (China, Japan, and Siberia) are different from those seen in Europe [11,12]. This may partly be explained by the finding that the species of *Ixodes* ticks commonly found in Asia (*Ixodes persulcatus* and *Ixodes ovatus*) harbour different genotypes from the European *Ixodes ricinus* [11]. In addition, three clusters of *C.* Neoehrlichia mikurensis were identified in China, on the basis of phylogenetic analyses of bacterial DNA recovered from rodents, ticks, and infected humans [12].

## Vectors, reservoirs, and transmission

Seven species of ticks have been reported to be infected by *C.* Neoehrlichia mikurensis: *I. ricinus* [48]; *I. persulcatus* [5,15]; *I. ovatus* [1]; *Ixodes frontalis* [57]; *Ixodes hexagonus* [30]; *Dermacentor reticulatus* [29,30]; and *Haemaphysalis concinna* [13]. It should be emphasized that the infection rates of *C.* Neoehrlichia mikurensis are highest among the *Ixodes* species, indicating that these are the primary vectors; the importance of the other tick genera as vectors is disputable, as they rarely carry *C.* Neoehrlichia mikurensis (Table 1).

The life stage of the tick does not seem to influence the infection rate of *C.* Neoehrlichia mikurensis. Whereas one study showed that nymphs were twice as likely to be infected by *C.* Neoehrlichia mikurensis as were adult ticks [28], the opposite pattern was seen in another study [31], and no difference in rates of infection between the life stages was observed in a third study [49]. *C.* Neoehrlichia mikurensis-infected ticks have been

**TABLE 1. Estimated prevalence rates of *Candidatus* Neoehrlichia mikurensis in ticks worldwide**

| | Ticks | | | | |
|---|---|---|---|---|---|
| **Country** | **Species** | **Source** | **No. investigated (pooled)[a]** | **Prevalence (%)** | **References** |
| Austria | *Ixodes ricinus* | Questing | 19/86 | 22 | [18] |
| | *I. ricinus* | Questing | 22/518 (10) | 4.2 | [26] |
| Belgium | *I. ricinus* | Questing | 6/373 | 1.6 | [43] |
| China | *Ixodes persulcatus* | Questing | 6/316 | 1.9 | [13] |
| | *Haemaphysalis concinna* | Questing | 2/187 | 0.8 | [13] |
| Czech Republic | *I. ricinus* | Questing | 3/138 | 2.2 | [18] |
| | *I. ricinus* | Questing | 54 pools (3–5) | 0.4–4.4 | [17] |
| | *I. ricinus* | Questing | 2/20 | 10 | [28] |
| France | *I. ricinus* | Questing | 1/60 | 1.7 | [28] |
| Germany | *I. ricinus* | Wild boars | 1/16 | 6.2 | [32] |
| | *I. ricinus* | Dogs | 32/773 | 4.1 | [30] |
| | *Ixodes hexagonus* | Dogs | 10/151 | 6.6 | [30] |
| | *Dermacentor reticulatus* | Questing | 1/1237 | 0.08 | [30] |
| | *I. ricinus* | Questing | 13/192 | 6.8 | [27] |
| | *D. reticulatus* | Questing | 0/283 | 0 | [27] |
| | *I. ricinus* | Questing | 44/542 | 8.1 | [28] |
| | *I. ricinus* | Humans | 9/111 | 8.1 | [28] |
| | *I. ricinus* | Rodents | 32/918 | 3.5 | [29] |
| | *D. reticulatus* | Rodents | 1/40 | 2.5 | [29] |
| | *I. ricinus* | Questing | 51/2315 | 2.2 | [29] |
| Hungary | *I. ricinus* | Questing | 3/34 | 8.8 | [25] |
| | *D. reticulatus* | Questing | 0/64 | 0 | [25] |
| | *H. concinna* | Questing | 0/62 | 0 | [25] |
| Italy | *I. ricinus* | Humans | 10/357 | 2.8 | [7] |
| | *I. ricinus* | Humans | 2/64 | 3.1 | [46] |
| | *I. ricinus* | Questing | 20/193 | 10 | [44] |
| Moldova | *I. ricinus* | Questing | 1/126 | 0.79 | [22] |
| The Netherlands | *I. ricinus* | Roe deer | 8/121 | 6.6 | [3] |
| | *I. ricinus* | Questing | 21/180 | 12 | [48] |
| | *I. ricinus* | Humans | 31/289 | 11 | [59] |
| | *I. ricinus* | Questing | 300/5343 | 5.6 | [55] |
| | *I. ricinus* | Questing | 160/2002 | 8.0 | [43] |
| | *I. ricinus* | Red deer | 26/409 | 6.4 | [43] |
| | *I. ricinus* | Wild boar | 4/84 | 8.3 | [43] |
| | *I. ricinus* | Sheep | 33/264 | 12 | [43] |
| | *I. ricinus* | Mouflon | 10/233 | 4.3 | [43] |
| Norway | *I. ricinus* | Questing | 8/341 | 2.3 | [2] |
| Poland | *I. ricinus* | Questing | 3/1325 (10) | 0.23 | [38] |
| | *I. ricinus* | Questing | 0/40 | 0 | [28] |
| Portugal[b] | *I. ricinus* | Questing | 0/101 | 0 | [28] |
| Russia | *I. persulcatus* | Questing | 2/53 | 3.8 | [5] |
| | *I. ricinus* | Questing | 21/295 | 7.1 | [4] |
| | *Ixodes frontalis*, *I. ricinus* | Birds | 2/139 | 1.4 | [57] |
| | *I. persulcatus* | Questing | 8/3552 | 0.22 | [15] |
| | *I. persulcatus* | Questing | 5/2590 | 0.19 | [16] |
| Slovakia | *I. ricinus* | Questing | 47/1311 | 3.6 | [18] |
| | *I. ricinus* | Questing | 16/670 | 2.4 | [21] |
| | *I. ricinus* | Questing | 2/68 | 2.9 | [19] |
| Spain | *I. ricinus* | Cows | 2/200 | 1.0 | [47] |
| Sweden | *I. ricinus* | Questing | 57/949 | 6.0 | [49] |
| Switzerland | *I. ricinus* | Birds | 7/215 | 3.3 | [37] |
| | *I. ricinus* | Questing | 1916 (5–10) | 3.5–8.0 | [36] |
| | *I. ricinus* | Questing | 52/818 | 6.4 | [33] |
| UK | *I. ricinus* | Questing | 0/954 | 0 | [54] |
| | *D. reticulatus* | Questing | 0/61 | 0 | [53] |
| | *Haemaphysalis punctata* | Questing | 0/100 | 0 | [53] |
| | *I. ricinus* | Various | 0/338 | 0 | [43] |
| | *D. reticulatus* | Various | 0/63 | 0 | [43] |

[a]Number of ticks per pool.
[b]Madeira Island.

collected from birds and a variety of mammals (Table 1), all of which may facilitate the spread of the infection in the environment.

Transmission of *C.* Neoehrlichia mikurensis to humans is surmised to occur via tick bites, as ticks infected with similar or identical sequence variants have been found in the surroundings of infected persons [11,13,36]. Although many patients recall specific tick bites [10], this is not always the case [10], as is typical for tick-borne infectious agents [58]. Studies on the prevalence of *C.* Neoehrlichia mikurensis in ticks removed from people in The Netherlands [59] and Germany [28] have estimated that every ninth to 12th tick bite represents a risk of transmission of *C.* Neoehrlichia mikurensis, although the risk of becoming infected is likely to be considerably lower [59]. Infected ticks and rodents have been found in the vicinity of large cities, such as Berlin, Budapest, Guangzhou, Leipzig, and Zurich, indicating that large populations may be exposed to this new infectious agent [6,24,30,31,36,60].

Until 2014, *C.* Neoehrlichia mikurensis had not been found in the larval stage of ticks [43], and it had been inferred that it is not transmitted transovarially [30]. However, it was recently reported that four of ten larvae sampled in Austria were

 

**TABLE 2. Estimated prevalence rates of *Candidatus* Neoehrlichia mikurensis in rodents worldwide**

| | Rodents | | | | |
|---|---|---|---|---|---|
| **Country** | **Species** | **Common name** | **No. investigated** | **Prevalence (%)** | **References** |
| China | *Rattus norvegicus* | Brown rat | 3–4/15 | 20–27 | [6] |
| | *Apodemus agrarius* | Striped field mouse | 14/117 | 12 | [12] |
| | *Apodemus sylvaticus* | Wood mouse | 5/40 | 12 | [12] |
| | *Apodemus draco* | South China field mouse | 1/7 | 14 | [12] |
| | *Apodemus peninsulae* | Korean field mouse | 5/57 | 8.8 | [12] |
| | *Eothenomys custos* | Southwest China vole | 2/8 | 25 | [12] |
| | *Myodes rufocanus* | Grey red-backed vole | 4/83 | 4.8 | [12] |
| | *Niviventer confucianus* | Chinese white-bellied rat | 1/52 | 1.9 | [12] |
| | *R. norvegicus* | Brown rat | 1/87 | 1.1 | [12] |
| | *Tamias sibiricus* | Siberian chipmunk | 1/7 | 14 | [12] |
| | *Clethrionomys rufocanus* | Grey red-backed vole | 5/109 | 4.6 | [13] |
| | *R. norvegicus* | Brown rat | 2/35 | 5.7 | [13] |
| | *T. sibiricus* | Siberian chipmunk | 1/3 | 33 | [13] |
| France | *Myodes glareolus* | Bank vole | 5/276 | 1.8 | [41] |
| Germany | *M. glareolus* | Bank vole | 16/56 | 29 | [30] |
| | *Microtus arvalis* | Common vole | 4/11 | 36 | [30] |
| | *Microtus agrestis* | Field vole | 2/2 | 100 | [30] |
| | *Apodemus flavicollis* | Yellow-necked mouse | 10/82 | 12 | [30] |
| | *Apodemus agrarius* | Striped field mouse | 4/78 | 5.2 | [30] |
| | *Apodemus flavicollis* | Yellow-necked mouse | 24/37 | 65 | [31] |
| | *Apodemus agrarius* | Striped field mouse | 1/3 | 33 | [31] |
| | *M. glareolus* | Bank vole | 23/42 | 55 | [31] |
| | *M. glareolus* | Bank vole | 125/396 | 32 | [29] |
| | *Apodemus sylvaticus* | Wood mouse | 1/36 | 2.8 | [29] |
| | *Apodemus flavicollis* | Yellow-necked mouse | 50/178 | 28 | [29] |
| | *Microtus arvalis* | Common vole | 4/7 | 57 | [29] |
| Hungary | *Apodemus flavicollis* | Yellow-necked mouse | 3/67 | 4.5 | [25] |
| | *Apodemus agrarius* | Striped field mouse | 3/92 | 3.3 | [25] |
| Italy | *Clethrionomys glareolus* | Bank vole | 1/34 | 2.9 | [45] |
| Japan | *Apodemus speciosus* | Large Japanese field mouse | 5/55 | 9.1 | [14] |
| | *Apodemus argenteus* | Small Japanese field mouse | 2/7 | 28 | [14] |
| | *R. norvegicus* | Brown rat | 7/15 | 47 | [1] |
| The Netherlands | *Apodemus sylvaticus* | Wood mouse | 5/23 | 22 | [43] |
| | *Microtus arvalis* | Common vole | 2/8 | 25 | [43] |
| | *M. glareolus* | Bank vole | 4/35 | 11 | [43] |
| Russia | *M. rufocanus* | Grey red-backed vole | 1/606 | 0.17 | [16] |
| | *Apodemus peninsulae* | Korean field mouse | 3/236 | 1.3 | [16] |
| | *Microtus* spp. | Vole | 1/38 | 2.6 | [16] |
| Slovakia | *Apodemus* spp., *M. glareolus* | Mice and voles | 31/286 | 11 | [20] |
| | *Apodemus* spp., *C. glareolus* | Mice and voles | 0/30 | 0 | [19] |
| Sweden | *M. glareolus* | Bank vole | 50/261 | 19 | [56] |
| | *M. glareolus* | Bank vole | 64/705 | 9.1 | [50] |
| | *Microtus agrestis* | Field vole | 2/24 | 8.3 | [50] |
| | *Apodemus sylvaticus* | Wood mouse | 1/10 | 10 | [50] |
| | *Apodemus flavicollis* | Yellow-necked mouse | 1/25 | 4.0 | [50] |
| Switzerland | *Apodemus* spp., *M. glareolus* | Mice and voles | 8/100 | 8.0 | [34] |

infected with *C.* Neoehrlichia mikurensis [18], implying the possibility of transmission of *C.* Neoehrlichia mikurensis from one generation of ticks to another; alternatively, the larvae may have become infected after interrupted feeding on an infected rodent. Whether transovarial transmission exists is still an open question, and it is generally assumed that *C.* Neoehrlichia mikurensis is dependent on a reservoir host for its survival. Burri *et al.* have proven that wild rodents are competent hosts for *C.* Neoehrlichia mikurensis, by showing that seven of eight naturally infected wild rodents could transmit the infection to pathogen-free ticks reared in the laboratory [34]. Moreover, they demonstrated double transmission of infection, in that one rodent was shown to transmit both *Borrelia afzelii* and *C.* Neoehrlichia mikurensis to the laboratory ticks.

Most species of wild rodents have the potential to serve as hosts for *C.* Neoehrlichia mikurensis. This role has been described for infected voles, field mice, wood mice, rats, and even chipmunks, but not for shrews [1,12,31,50]. The prevalence rates of *C.* Neoehrlichia mikurensis infection among rodents trapped in various parts of the world are shown in Table 2. Hedgehogs are also potential reservoirs of *C.* Neoehrlichia mikurensis, although their capacity to act as true reservoirs of infection has not been tested [24]. If one compares the prevalence rates of *C.* Neoehrlichia mikurensis in ticks (Table 1) and in rodents (Table 2), it can be seen that they are approximately two-fold higher among rodents. This has been interpreted to support the concept that rodents are competent hosts for *C.* Neoehrlichia mikurensis, perhaps being required for the survival of *C.* Neoehrlichia mikurensis in the environment [31].

Kawahara *et al.* demonstrated rodent-to-rodent transmission of *C.* Neoehrlichia mikurensis infection through intraperitoneal injections of spleen homogenates from infected wild rats into laboratory rats [1]. The first evidence of *C.* Neoehrlichia mikurensis infection was seen after 3 weeks (weak detection of the pathogen in spleen samples with nested PCR). After 2

months, the rats were unequivocally infected, showing strong *C.* Neoehrlichia mikurensis-specific PCR reactivity in the spleen, liver, and blood, without the need for nested PCR. The same group failed to infect mice by inoculating *C.* Neoehrlichia mikurensis-positive tick homogenates, although it is likely that the mice were killed too early (after 10 days) for the infection to be detected [1].

*C.* Neoehrlichia mikurensis is mainly detected in the spleen, liver and kidneys of rodents, i.e. organs that are involved in the clearance of systemic bacteria [1,12,25,43]. *C.* Neoehrlichia mikurensis has also been detected in the brain and skin of rodents [25,31,43]. There are no accounts of experimentally infected rats being ill [1], and trapped, infected wild rodents have not shown any apparent signs of disease (M. Andersson, personal communication). In addition, rodents seem to clear the infection within a few months, and are therefore not lifelong carriers of the infection [56]. However, a recent study showed that fetuses and newborn pups of *C.* Neoehrlichia mikurensis-infected wild rodents tested positive for *C.* Neoehrlichia mikurensis, suggesting that transplacental transmission of infection may occur [29]. Interestingly, rodents are refractory to infection with the other *Neoehrlichia* species, *C.* Neoehrlichia lotoris [8].

Apart from humans, the dog is the only animal known to develop symptomatic *C.* Neoehrlichia mikurensis infection. This is based on a single case report of an 8-year-old female Irish Setter in Germany that was diagnosed with *C.* Neoehrlichia mikurensis infection when she developed coagulopathy after surgery for suspected mammary carcinoma [61]. The dog had moderate thrombocytopenia but normal levels of coagulation factors, and she subsequently became neutropenic [61]. Two courses of antibiotics were required to clear the infection. It was suggested that the dog was an asymptomatic carrier of *C.* Neoehrlichia mikurensis, and that the active infection was triggered by surgery-induced lowering of immune defence mechanisms [61].

## Human *C.* Neoehrlichia mikurensis infection

The first reports of *C.* Neoehrlichia mikurensis infection in humans in Europe were published in 2010 [11,35,52], 11 years after the bacterium was first described in ticks [3]. Although human infection does not fulfill Koch's Postulates, as the bacterium has not yet been cultivated, there are three compelling lines of evidence for an aetiological linkage between the presence of *C.* Neoehrlichia mikurensis DNA in patient samples and the development of infectious disease in immunocompromised patients [62]: (a) high numbers of bacterial gene copies are seen in the blood samples of diseased persons; (b) the infectious disease resembles anaplasmosis/ehrlichiosis caused by related species; and (c) targeted antibiotic therapy results in the resolution of symptoms and clearance of bacterial gene copies in the bloodstream within days.

The clinical picture of neoehrlichiosis, which is the term proposed to designate human infection with *C.* Neoehrlichia mikurensis, is rather dramatic in immunocompromised patients (Table 3). A high and remitting fever, which is often accompanied by chills and nightly sweats, is a characteristic finding [10]. In many cases, there is severe pain, which may be localized and/or migrating, and that may affect the neck, temporal/mandibular joints, elbows, knees and ankles, muscles of the trunk, or extremities. Skin rashes that resemble erysipelas or erythema nodosum may develop. Less specific symptoms, such as cough, diarrhoea, and weight loss resulting from protracted systemic inflammation, may also occur (Table 3).

There is a remarkably high incidence of vascular and thromboembolic events among the clinical signs of neoehrlichiosis in patients in different European countries (Table 3). These events may occur in the venous part of the circulation, and may be manifested as deep vein thrombosis of the extremities and/or pulmonary embolism. However, thromboembolic events occurring in the arteries, e.g. transient ischaemic attacks, which give rise to mental confusion, weakness and numbness, are also seen. Ankle oedema and tender subcutaneous veins are also noted, and may reflect vascular reactions. It is not known whether these thromboembolic complications result from inflamed and/or infected blood vessels [10].

Although neoehrlichiosis afflicts immunocompromised individuals, it is premature to designate *C.* Neoehrlichia mikurensis as an opportunistic pathogen. There are currently only two studies of *C.* Neoehrlichia mikurensis infection in non-immunocompromised persons [13,39] and two case reports [11,35], representing 14 patients in total (Table 4). It could be argued that one of these patients was immunocompromised because of major surgery [35], similarly to the case of canine *C.* Neoehrlichia mikurensis infection [61]. Of the more comprehensive studies on neoehrlichiosis in healthy subjects, one was a hospital-based study of summer fever in tick-exposed persons in north-eastern China [13], and the other was a surveillance study of extensively tick-exposed forestry workers in Poland [39].

The following host factors predispose patients to contracting more severe neoehrlichiosis (Table 3): advanced age; a disease that engages the adaptive immune system, either a haematological malignancy (malignant lymphoma or chronic lymphocytic leukaemia) or an autoimmune/rheumatic disease (rheumatoid arthritis, systemic lupus erythematosus, or psoriasis); and recent chemotherapy or corticosteroid treatment [10]. Splenectomy is

 

**TABLE 3.** Neoehrlichiosis in immunocompromised patients

| Demographic factors | | | | Host factors, no. (%) | | | | | Clinical picture, no. (%) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Country | Mean age in years (range) | F/M ratio | Tick-bitten, no. (%) | Haematological malignancy | Autoimmune disease | Lack spleen | Chemotherapy | Systemic corticosteroids | Rituximab | Fever | Myalgia/ arthralgia | Vascular/ thromboembolic events | Weight loss | Skin rash | References |
| Sweden, *n* = 6 | 64 (54–77) | 3/8 | 5/11 | 8/11 | 6/11 | 8/11 | 6/11 | 7/11 | 5/11 | 11/11 | 8/11 | 6/11 | 4/11 | 4/11 | [10,52] |
| Switzerland, *n* = 2 | | | | | | | | | | | | | | | [36] |
| Czech Republic, *n* = 2 | | | | | | | | | | | | | | | [9] |
| Germany, *n* = 1 | 71 | 1/0 | 1/1 | 0/1 | 1/1 | 0/1 | 1/1 | 0/1 | 1/1 | 1/1 | 0/1 | 0/1 | 1/1 | 0/1 | [11] |
| Sweden, *n* = 1 | | 67% M | 6/12 (50) | 8/12 (67) | 7/12 (58) | 8/12 (67) | 7/12 (58) | 7/12 (58) | 6/12 (50) | 12/12 (100) | 8/12 (67) | 6/12 (50) | 5/12 (42) | 4/12 (33) | [64] |
| Total, *n* = 12 | 65 | | | | | | | | | | | | | | |

F, female; M, male.

a strong predisposing factor for severe disease, with the majority of immunocompromised patients with neoehrlichiosis having undergone this procedure [10]. The importance of the spleen probably reflects its capacity to produce natural IgM antibodies, which constitute an important innate immune defence mechanism [63].

The significance of B-cells and/or antibodies in the host defence against *C.* Neoehrlichia mikurensis is reinforced by the finding that many patients with neoehrlichiosis have been treated with rituximab (Table 3), a monoclonal antibody directed against CD20 on B-cells [10,64]. The CD20 molecule is expressed by all stages of B-cells, with the exception of plasma cells, the producers of antibodies. Thus, rituximab-treated patients, including those with neoehrlichiosis, may present with normal levels of immunoglobulins in the serum [64]. Rituximab is used to treat malignant lymphomas and systemic rheumatic diseases. Physicians in charge of such patients in *C.* Neoehrlichia mikurensis-endemic regions should be alert to the development of thromboembolic events, as this may be a sign of neoehrlichiosis.

The clinical picture of *C.* Neoehrlichia mikurensis infection in immunocompetent individuals is more diverse (Table 4), ranging from asymptomatic infection [39] to febrile illness with a range of associated symptoms [13], and a possibly fatal outcome [11]. The prevalence of *C.* Neoehrlichia mikurensis infection in the Chinese study was 1.1% (seven of 622 patients); the rates of *C.* Neoehrlichia mikurensis infection among ticks and rodents in the same area were 1.6% and 3.8%, respectively [13]. In addition to fever, all of the patients in that study reported headache and malaise. Vomiting, nausea, myalgia and neck stiffness affected a majority of the patients, whereas arthralgia, cough, diarrhoea, confusion and erythema were observed less frequently (Table 4). Whether or not the patients were treated with antibiotics and how rapidly they cleared and recovered from the infection were not reported. A very similar rate of *C.* Neoehrlichia mikurensis infection was seen in the Polish study, i.e. 1.6% (five of 316 patients) [39]. In contrast to the participants in the Chinese study, all five individuals in the Polish study who had positive blood samples were asymptomatic (Table 4).

The incubation period between exposure to *C.* Neoehrlichia mikurensis and symptomatic infection is uncertain. Li *et al.* reported that the median time from tick bite to onset of illness in their patients was 8 days (range, 2–35 days) [13]. However, this is a gross estimate, as the ticks were not collected and analysed for the presence of *C.* Neoehrlichia mikurensis, and it is likely that these patients (all of whom were farmers) had been subjected to repeated and unnoticed tick bites [13].

Typical laboratory findings in immunocompromised patients with neoehrlichiosis are elevated white blood cell counts, neutrophilia, increases in the levels of inflammatory markers, such as

**TABLE 4.** ***Candidatus*** **Neoehrlichia mikurensis infection in immunocompetent patients**

| Demographic and host factors | | | | | | Clinical picture | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Country | No. of patients | F/M ratio | Age in years (range) | Other disease | Tick-bitten | Fever, no. (%) | Myalgia/ arthralgia, no. (%) | Skin rash, no. (%) | Vascular/thromboembolic events | References |
| Germany | 1 | 0/1 | 57 | No | 0/1 | 1/1 | 0/1 | 0/1 | Aneurysm of cerebral artery Cerebral haemorrhage and infarction | [11] |
| Switzerland | 1 | 0/1 | 61 | Post-CABG and mitral valve surgery | 0/1 | 1/1 | 0/1 | 0/1 | Prophylactic anticoagulant therapy | [35] |
| China | 7 | 2/5 | 41 (29–67) | No | 7/7 | 7/7 | 4/7 | 1/7 | Confusion in one patient | [13] |
| Poland | 5 | 1/4 | ND | No | 5/5 | 0/5 | 0/5 | 0/5 | No | [39] |
| Total | 14 | 79% M | | | 12/14 (86) | 9/14 (64) | 4/14 (29) | 1/14 (7) | | |

CABG, coronary artery bypass graft; F, female; M, male; ND, not described.

C-reactive protein, procalcitonin, and erythrocyte sedimentation rate [36], and anaemia [10]. Occasionally, modest thrombocytopenia, lymphopenia and hyponatraemia, slightly elevated levels of hepatic transaminases, and increased levels of lactate dehydrogenase are seen [10]. The two reports on immunocompetent patients show a similar profile, i.e. leukocytosis [11,35], neutrophilia [35], and raised serum C-reactive protein [11,35]. In contrast, Li *et al.* reported that five of seven of their patients had normal leukocyte counts, and that the remaining two patients had leukocytosis and leukopenia, respectively [13]. In addition, anaemia and thrombocytopenia occurred in two patients, although it was not specified whether these were the same patients who had altered leukocyte counts [13].

## Diagnostics

Although *C.* Neoehrlichia mikurensis was described in The Netherlands 15 years ago [3], and it is appreciated that every ninth Dutch tick bite carries the risk of *C.* Neoehrlichia mikurensis transmission [59], not a single case of neoehrlichiosis has been reported from that country. It is likely that cases have been missed because of diagnostic limitations. Patients diagnosed with neoehrlichiosis have, as a rule, been subjected to numerous investigations to find the cause of their fever, leading to considerable delays in diagnosis (median of 60 days) [10]. These patients have also, without exception, been treated unsuccessfully with various broad-spectrum antibiotics before the correct diagnosis has been reached [10].

Currently, the only diagnostic option for *C.* Neoehrlichia mikurensis infection is PCR. Three types of PCR have been used to detect *C.* Neoehrlichia mikurensis in human samples: pan-bacterial PCR directed against the 16S rRNA gene [11,35,52]; nested PCR specific for the 16S rRNA gene of *Anaplasmataceae* [13]; and *C.* Neoehrlichia mikurensis-specific PCR targeting the 16S rRNA gene or *gro*ESL gene [10,64]. The first two types of PCR require sequencing of the flanking gene segments. A multiplex PCR has been developed that incorporates probes specific for the *Anaplasmataceae* family, *Neoehrlichia* genus, and *C.* Neoehrlichia mikurensis, enabling the detection of all three targets in a single reaction [36]. Positive control plasmids that contain regions of the *C.* Neoehrlichia mikurensis 16S rRNA gene have been included in the PCRs [10,36]. These assays have shown that immunocompromised patients have high bacterial burdens, with the highest detected loads being $3 \times 10^7$ gene copies/mL in the bone marrow and $9 \times 10^6$ gene copies/mL in the blood of a splenectomized patient with chronic lymphocytic leukaemia [36].

Two types of human sample have yielded positive PCR results in neoehrlichiosis patients: blood (plasma, serum, whole blood, and blood culture flask contents) and bone marrow [10]. Even though mental confusion and other neurological symptoms have been observed in a few patients with neoehrlichiosis, *C.* Neoehrlichia mikurensis DNA has not been detected in the cerebrospinal fluids of such patients [10].

*C.* Neoehrlichia mikurensis has not been cultivated, its genome has not been sequenced, and its surface antigens remain unknown, so serological assays are not available. Although it is claimed that there is no serological cross-reactivity between *C.* Neoehrlichia mikurensis and *E. chaffeensis* and/or *A. phagocytophilum*, this is mainly based on studies of immunocompromised patients, who may be incapable of producing antibodies in response to infection. There is one report of an immunocompetent neoehrlichiosis patient who apparently developed IgM antibodies cross-reactive to *A. phagocytophilum*, which gave an atypical pattern in the indirect fluorescence assay that was employed, and a low IgG titre [35]. The patient tested negative in an *A. phagocytophilum* PCR [35].

## Treatment

Doxycycline is active against intracellular bacteria, and is recommended for the treatment of neoehrlichiosis. Oral

 

administration of 100 mg of doxycycline twice daily has been used for almost every published case of neoehrlichiosis. Rifampin (300 mg twice daily) was used successfully in a patient with suspected hypersensitivity to doxycycline (own unpublished observation), and another patient was treated successfully with a combination of rifampin (450 mg twice daily) and doxycycline (100 mg twice daily) [35]. The optimal length of time for which treatment should be given for neoehrlichiosis is not known. Most studies report a treatment duration of 3 weeks [10], although shorter (2 weeks) [64] and considerably longer (6 weeks) courses of treatment have been used [35]. Importantly, all known cases of immunocompromised patients with neoehrlichiosis who were treated with adequate antibiotics improved rapidly, with a median time to resolution of symptoms of 5 days, and all survived, with follow-up PCR analyses of their blood samples for *C.* Neoehrlichia mikurensis yielding negative results [10,64].

## Conclusion

Although much knowledge has been gained during the last few years, since we learned that *C.* Neoehrlichia mikurensis can cause human disease, much remains to be resolved. We need to learn how to cultivate it, to be able to sequence its genome and study host–pathogen interactions at the cellular level. Nothing is known regarding its virulence factors, and very little is known regarding its immunopathogenic mechanisms. Serological assays are needed to establish the degree of exposure of people (and dogs) to this new pathogenic species, and determine its impact on public health. The field of *C.* Neoehrlichia mikurensis research is wide open, and will hopefully reveal many exciting findings in the near future.

## Transparency declaration

The author declares that she has no conflicts of interest.

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